constructs are to be transferred to sexually incompatible relatives tissue culture techniques such as wide crosses and/or embryo rescue may be employed. A variety of techniques known to those skilled in the art may be employed to derive the combination of repressible lethal and repressor genes which provides the greatest utility within the scope of the present invention.

The following examples are set forth to illustrate the method and in no way limit the scope of the invention.

Example 1: Isolation of oncogene 1 and 2 from *Agrobacterium* Ti-plasmid pTi15955.

To isolate the oncogenes, the following steps were employed. The subclones p101 and p202, detailed in US 5,428,147 encompassing the DNA encoding oncogene 1 (p202) and oncogene 2 (p101) are used as a source of the genes. In order to isolate the genes, a combination of PCR to introduce convenient restriction sites and subcloning of native gene fragments is employed to derive oncogenes that can be conveniently inserted into plant transformation vectors.

To isolate a native oncogene 2, the following approach is used. The 5' region, including the native promoter of oncogene 2 is isolated by PCR amplification of the plasmid p101 with the following primers:

G2P1 (SEQ ID NO.1)

5'ATA**GCATGC**TCTAGATGTTAGAAAAGATTCGTTTTTGTG 3'

and, G2P2 (SEQ ID NO:2)

5' ATA**CCATGG**CGATCAATTTTTTTGGCGC 3'

G2P1 contains a Sph 1 site (boldface) and a Xba I site (underlined) and corresponds to the complement of nucleotides 5808 - 5785 in the published sequence of pTi15955. G2P2 contains a Nco 1 site (boldface) and corresponds to nucleotides 5285 - 5309 in the published sequence of pTi15955. The use of G2P1 and G2P2 yields a fragment of 523 bp which represents the 5' region of the native oncogene 2, including the promoter modified to contain a Sph 1 and Xba I site at the 5' end of the promoter.

To isolate the 3' region of oncogene 2, including the native terminator structure, two PCR primers are used. The first primer used is:

G2P3 (SEQ ID NO:3)

5' ATA**AAGCTT**GAAAATTAAGCCCCCCCCCG 3'

and, G2P4 (SEQ ID NO:4)

5' ATAGGATCC**GCATGCC**CAGTCTAGGTCGAGGGAGGCC 3'

G2P3 contains a Hind III site (boldface) and corresponds to the complement of nucleotides 3396 - 3371 of the published sequence of pTi 15955. G2P4 contains a Sph 1 site (boldface) and a Bam H1 site (underlined) and corresponds to nucleotides 3237 - 3264 of the published sequence of pTi 15955. The use of G2P3 and G2P4 yields a fragment of 164 bp which represents a portion of the 3' end of the native oncogene 2.

The plasmid p101 is digested with Nco I and Hind III to yield a fragment of approximately 1895 bp fragment of

oncogene 2 which encompasses most of the coding region. The 523 bp fragment of the 5' end of the native oncogene 2 is digested with Nco I and ligated to the Nco I site of the 1895 bp fragment and the 164 bp 3' end of the gene is digested with Hind III and ligated to the Hind III site of the 1895 bp fragment. The reconstructed native oncogene 2 is then digested with Sph 1 and subcloned into the Sph 1 site of the common cloning vector pGEM-4Z (Promega, La Jolla, California). This vector is called pG2. DNA sequencing was used to verify the composition of this reconstructed DNA corresponding to the authentic DNA sequence of the native oncogene 2.

Isolation of oncogene 1 employs a combination of PCR to introduce convenient restriction sites and subcloning of a native gene fragment. To isolate the required fragments, the following approach is used. Convenient restriction sites at the 5' end of the coding region are introduced by PCR, employing the following two primers:

G1P1 (SEQ ID NO:5)

5' ATA**ATCGATAT**AGAAACGGTTGTTGTGGTT 3'

and, G1P2 (SEQ ID NO:6)

5' ATA**AGATCT**CGGGGAAGCGACC 3'

G1P1 contains a Cla 1 site (boldface) and corresponds to nucleotides 5755 - 5775 of the published sequence of pTi 15955. G1P2 contains a Bgl II site (boldface) and corresponds to the complement of nucleotides 6028 - 6010 of the published sequence of pTi 15955. G1P1 and G1P2 are used to amplify a 273 bp fragment of oncogene 1 which is modified to contain a Cla 1 site at the 5' end of the coding region.

To isolate a 3' fragment of the coding region of oncogene 1, two primers are used to introduce convenient restrictions sites at the 3' end of the coding region.

G1P3 (SEQ ID NO:7)

5' AAT**GATATC**TGAACTTTATGATAAGG 3'

and, G1P4 (SEQ ID NO:8)

5' ATAGAGCTC**ATCGAT**ACTAATTTCTAGTGCGGTAGTT 3'

G1P3 contains a Eco RV site (boldface) and corresponds to nucleotides 7350 - 7372 of the published sequence of pTi 15955. G1P4 contains a Cla 1 site (boldface) and a Sac 1 site (underlined) and corresponds to nucleotides 8076 - 8056 of the published sequence of pTi 15955. The use of G1P3 and G1P4 results in a 732 bp fragment representing the 3' end of the coding region of oncogene 1.

In order to reconstruct a complete coding region of the oncogene 1, the plasmid p202 is digested with Bgl II and the 1697 bp fragment encompassing the partial coding region of the oncogene 1 is isolated. To the 5' end of

containing three copies of the tet operator sequence. The means by which this is accomplished is as follows and is shown in Figure 8.

The promoter region of the phaseolin gene (described in: Slightom, J.L., Sun, S.M. and Hall, T.C., Proc. Natl. Acad. Sci. USA 80:1897-1901, 1983) is isolated by PCR using the vector pAGM 219, kindly supplied by Dr. G. Cardineau of Mycogen Plant Sciences, San Diego, California. The plasmid pAGM 219 contains approximately 1600 base pairs of the promoter region of the phaseolin gene and the native termination region of the phaseolin gene. The region of the promoter 5′ to the TATA box was isolated by PCR in preparation for the addition of a synthetic DNA sequence comprising the tet operator DNA and a TATA box.

The first PCR primer used was engineered to introduce a Csp45 1 site by a minor alteration of the nucleotide sequence in the native promoter sequence. The sequence of this primer is shown below:

SEQ ID NO:9

5'GGTGG**TTCGAA**CATGCATGGAGATTTG 3'

The Csp45 1 restriction site is shown in boldface. The second primer used for PCR has the following sequence:

SEQ ID NO:10

5'CCGTAT**CTCGAG**ACACATCTTCTAAAGTAATTT 3'

A Xho 1 site is indicated in boldface. The PCR product obtained using these primers was called pPHAS and corresponds to nucleotides 128 - 833 of the DNA sequence of the phaseolin promoter of the lambda genomic clone AG-λPVPh177.4 (λ177.4), (Slightom, J.L., Sun, S.M. and Hall, T.C., Proc. Natl. Acad. Sci. USA 80:1897-1901, 1983). A synthetic tet operator sequence was added to this fragment by joining the synthetic duplex DNA to the Csp45 1 site in the PCR product. The synthetic operator DNA sequence also comprises a Cla 1 site at the 3' end of the sequence. The top strand of the synthetic DNA has the following sequence:

SEQ ID NO:11

5'*TTCGAA*GACTCTATCAGTGATAGAGTGTATATAAGACTCTATCAGTGATAG AGTGAACTCTATCAGTGATACAGTATA*TCGAT* 3'

Which comprises 3 copies of the operator DNA (boldface), a TATA box (underlined), a Csp45 1 site at the 5' end (italics and underlined) and a Cla 1 site at the 3' end (italics and boldface). A bottom strand fragment is used which has the following sequence:

SEQ ID NO:12

5'CGATATACTGTATCACTGATAGAGTTCACTCTATCACTGATAGAGTCTTAT ATACACTCTATCACTGATAGAGTCTTCGTT 3'

Which comprises a complementary strand to SEQ ID NO:9 and contains a Cla 1 cohesive end, identified in boldface. The duplex DNA is referred to a "top" DNA and is ligated to the Csp45 1 and Cla 1 cut pPHAS and clones containing the inserted "top" DNA are chosen. This vector